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Pathogenesis of cerebral edema after treatment of diabetic ketoacidosis

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Pathogenesis of cerebral edema after treatment of diabetic ketoacidosis. We studied the roles of acidosis, plasma osmolality, and organic osmolytes in the pathogenesis of cerebral edema in an animal model of diabetes mellitus. Normonatremic rats with streptozotocin-induced nonketotic (NKD) and ketotic (DKA) diabetes were sacrificed before or after treatment with hypotonic saline and insulin. Brains were analyzed for water, electrolyte, and organic osmolyte content. Brain water decreased by 2% in untreated DKA and NKD despite a 12% increase in plasma osmolality due to hyperglycemia. After treatment of both NKD and DKA, brain water increased equivalently by 8%. The cerebral edema that occurred after treatment was associated with decreased brain sodium content and no change in total major brain organic osmolytes in both NKD and DKA. However, brain content of the individual osmolytes glutamine and taurine increased after treatment of DKA. In a separate study, brain water and solute content of rats with DKA were compared after treatment with either hypotonic or isotonic fluid. Animals treated with isotonic fluid had significantly less cerebral edema and higher brain sodium content than those treated with hypotonic fluid. In our studies, brain swelling after treatment of DKA and NKD was primarily due to a rapid reduction of plasma glucose and osmolality, and was not caused by sodium movement into the brain. Acidosis did not appear to play a major role in the pathogenesis of cerebral edema after treatment of DKA.

Cerebral edema after treatment of diabetic ketoacidosis (DKA) is an important cause of death in children with insulin-dependent diabetes mellitus [1–3]. Clinical studies have implicated a variety of factors that predispose to cerebral edema, including degree of acidosis and hyperglycemia, changes in plasma osmolality or oncotic pressure, and hyponatremia [4–7]. The relative contribution of these factors to the pathogenesis of brain swelling remains unclear. There is also disagreement over whether use of hypotonic replacement fluid in the treatment of DKA predisposes to cerebral edema [8, 9].

Cerebral edema has rarely been reported during treatment of the hyperosmolar nonketotic state in adults with noninsulin-dependent diabetes mellitus [10–12]. To explain why brain swelling is primarily a complication of ketoacidosis, van der Meulen, Klip and Grinstein proposed that correction of acidosis in DKA could predispose to cerebral edema by stimulation of the brain $Na^+/H+$ exchanger [13]. They suggest that acidification of the cytosol by organic ketoacids activates the plasma membrane $Na^+/H+$ exchanger, increasing brain sodium and water. Relative alkalinization of the extracellular fluid due to insulin treatment would further promote Na^+/H^+ exchange, favoring sodium and water influx into brain. Insulin, which activates Na^+/H^+ exchange in other tissues [14, 15], and hyperglycemia, which may cause osmotic disruption of the blood brain barrier [16], have also been proposed as pathogenic factors. These mechanisms would result in an influx of sodium and water into the brain.

An alternative suggestion is that the brain accumulates osmotically active solutes in DKA in an adaptive response to severe hyperglycemia. The proposed response would preserve cell volume but cause water flow into the brain as plasma osmolality is rapidly decreased during treatment. In this case, an adaptive efflux of sodium from brain would be expected.

Several experimental studies have attempted to define the pathogenic mechanism of cerebral edema after treatment of DKA. In hyperglycemic dogs, Prokop identified an increase in cerebrospinal fluid sorbitol and fructose content. He postulated that an increase in these solutes in brain resulted in brain edema when blood sugar was rapidly lowered [17]. Arieff and colleagues, in a series of studies in the early 1970s, induced severe hyperglycemia by glucose infusion in both normal and diabetic rabbits [18, 19]. They observed that two hours of hyperglycemia resulted in brain dehydration, but after four hours brain water had returned to normal values and measured brain osmolytes only partially accounted for an increase in total brain tissue osmolality as measured by freezing point osmometry. An increase in brain sorbitol or other measured osmolytes was not confirmed, and formation of "idiogenic osmoles" to preserve cell volume was proposed. Rapid correction of hyperglycemia with insulin caused sodium and chloride influx into the brain, a further increase in idiogenic osmoles, and cerebral edema. Lowering of glucose without insulin (by peritoneal dialysis) prevented the increase in brain sodium and water. Thus, it was suggested that insulin played a primary role in the pathogenesis of brain swelling by increasing brain sodium content and stimulating formation of unidentified solute. However, acidosis and plasma sodium were not controlled for in these studies.

Recently, it has been proposed that idiogenic osmoles involved in cell volume regulation are, in fact, organic osmolytes consisting primarily of amino acids, trimethylamines, and polyols [20, 21]. Study of changes in these brain osmolytes in diabetic ketoacidosis and its treatment has been limited. Harris et al found that taurine was increased in experimental DKA in rats, implying that this osmolyte also contributed to cell volume regulation in severe

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diabetes [22]. Trachtman, Futterweit and Sturman found an increase in cerebral synaptosomal taurine transport in diabetic rats [23], and Bedford and Leader described increases in rat brain content of taurine, glutamate, and glutamine after five days of hyperglycemia [24]. It has also been suggested that myoinositol accumulation might contribute to the cerebral edema after treatment of diabetes [25].

The current studies examine the roles of acidosis, plasma osmolality, and brain organic osmolytes in the pathogenesis of cerebral edema after treatment of DKA. We compare brain water and solute content in rat models of nonketotic and ketotic diabetes mellitus, and address the effect of hypotonic fluid administration during treatment of diabetic ketoacidosis on the development of cerebral edema.

Methods

For all studies, male Sprague-Dawley rats (Holtzman, Indianapolis, IN, USA) weighing 300 to 350 g were used. Rats were housed in 12-hour light-dark cycles and given free access to regular laboratory chow.

Experiment I: Treatment of non-ketotic versus ketotic diabetes

Rat model of untreated and treated nonketotic diabetes mellitus (NKD). Rats were divided into three experimental groups: Nondiabetic Controls, Untreated NKD, and Treated NKD. NKD was produced by injection of streptozocin (Upjohn, Kalamazoo, MI, USA) 65 mg/kg via superficial femoral vein under methohexital anesthesia (50 mg/kg i.p.). Nondiabetic control rats were similarly anesthetized and injected with citrate buffer intravenously. After recovery from anesthesia, all streptozocin injected rats were given D_{10} as drinking water for 24 hours following injection and tap water thereafter. Serial urine dipsticks (Ketosticks) were performed to ensure the absence of ketonuria in all rats. No maintenance insulin was administered following injection with streptozocin. Six days after the injection, all rats were anesthetized with ketamine (40 mg/kg i.p.) and PE-50 tubing was placed in the femoral vein and artery. Following line placement and initial blood sampling, Nondiabetic Control and Untreated NKD rats were sacrificed by decapitation and brain tissue was analyzed as described below.

Pretreatment blood samples were obtained from the treated NKD group, followed by a 60-minute intravenous infusion of regular insulin and hypotonic electrolyte solution. Intravenous fluid consisted of 42 mM NaCl, 30 mM $NaHCO_3$, and 5 mM KCl given as an initial bolus of 7.5 ml/100 g body wt over 15 minutes, followed by the continuous infusion of 2.5 ml/100 g body wt over the remaining 45 minutes of the 60-minute treatment period. Regular insulin was given as an initial bolus of 1.0 unit, followed by the continuous infusion in the intravenous fluid of 0.5 units over 15 minutes, and another 0.5 units/ml intravenous fluid over the remaining 45 minutes of the treatment period. Electrolyte content of the infusate was chosen to maintain a constant plasma sodium over the course of the treatment protocol. Sixty minutes after completing the treatment infusion and 120 minutes after beginning treatment, final blood sampling was performed and rats were sacrificed by decapitation.

Rat model of untreated and treated ketotic diabetes mellitus (DKA). Three groups of rats were studied: Nondiabetic Controls, Untreated DKA, and Treated DKA. The animals were anesthetized with methohexital as above and injected intravenously with streptozotocin 150 mg/kg. Nondiabetic Controls were injected with citrate buffer. After recovery from anesthesia all streptozocin injected rats were given D_{10} as drinking water for 24 hours, then switched to tap water. On days 2 through 5, a once-daily injection of Ultralente insulin 2 units was given subcutaneously to all DKA rats. This dose of insulin resulted in significant ketoacidosis with hyperglycemia. On day 6, rats were anesthetized with ketamine 40 mg/kg i.p. and arterial and venous lines were placed as in the NKD study. Following line placement and initial blood sampling, Nondiabetic Controls and Untreated NKD rats were sacrificed by decapitation and brain tissue was analyzed as described below.

Pretreatment blood samples were obtained from the treated DKA group, followed by a 60-minute intravenous infusion of regular insulin and hypotonic electrolyte solution. Intravenous fluid consisted of 75 mM $NaHCO_3$, and 25 mM KCl given as an initial bolus of 7.5 ml/100 g body wt over 15 minutes, followed by the continuous infusion of 3.5 ml/100 g body wt over the remaining 45 minutes of the 60-minute treatment period. Regular insulin was given as an initial bolus of 1.0 unit, followed by the continuous infusion in the intravenous fluid of 0.5 units over 15 minutes, and another 0.375 units/ml intravenous fluid over the remaining 45 minutes of the treatment period. Electrolyte content and rate of the infusate was chosen to maintain a constant plasma sodium over the course of the treatment protocol, and to correct metabolic acidosis. Sixty minutes after completing the treatment infusion and 120 minutes after beginning treatment, final blood sampling was performed and rats were sacrificed by decapitation.

Experiment II: Treatment of DKA with hypotonic versus isotonic fluid

This experiment examined the role of the osmolality of intravenous fluid used in treatment on the development of cerebral edema in DKA. DKA was induced as described in experiment I. Six days after injection with streptozotocin, two groups of DKA rats were studied. In one group, DKA was treated identically to that described in the DKA protocol in experiment I; in the second group, insulin treatment and the volume of infusate were identical to experiment I, but the electrolyte content was adjusted to be isotonic (75 mM $NaHCO_3$, 54 mM NaCl, and 25 mM KCl). Sixty minutes after completing the treatment infusion and 120 minutes after beginning treatment, rats were sacrificed by decapitation.

Analytical procedures

Plasma electrolytes, urea nitrogen, creatinine, glucose, total CO_2, and plasma osmolality were measured with a Synchron CX3 Clinical System (Beckman Instruments, Fullerton, CA, USA) and arterial blood gases with a Radiometer ABL 330 blood gas analyzer (Copenhagen, Sweden). Plasma β-hydroxybutyrate was measured with a β-hydroxybutyrate dehydrogenase enzymatic assay (Sigma, St. Louis, MO, USA).

Immediately after decapitation, rat skulls were bisected sagittally with an electric scroll saw. One hemisphere was quickly removed, placed in liquid nitrogen within 15 seconds, and stored at −70°C until analyzed for organic osmolyte content. The remaining hemisphere was removed and utilized for determination of water and electrolyte content. Freezing of brain *in situ*, required for accurate measurement of brain glucose, was not performed because this method would preclude precise measurement of brain water. Water content was determined by obtaining

Table 1. Plasma values and body weight in non-ketotic diabetic (NKD) and ketotic diabetic (DKA) rats

	Control $N = 9$	NKD untreated $N = 10$	NKD pre-treatment $N = 9$	NKD post-treatment $N = 9$	Control $N = 9$	DKA untreated $N = 11$	DKA pre-treatment $N = 11$	DKA post-treatment $N = 11$
Urea *mM*	5.2 ± 0.4	9.5 ± 0.7[a]	9.0 ± 0.4[a]	9.7 ± 0.6[a]	6.2 ± 0.4	13.2 ± 1.4[ab]	14.9 ± 2.6[a]	11.8 ± 2.3
Glucose *mM*	9.9 ± 0.5	43.7 ± 2.2[a]	41.6 ± 2.9[a]	6.4 ± 0.6[c]	9.6 ± 0.4	31.3 ± 0.8[ab]	32.1 ± 1.5[a]	6.2 ± 0.5[ad]
Osmolality *mOsm/kg*	305 ± 3	345 ± 6[a]	352 ± 2[a]	309 ± 5[c]	301 ± 3[b]	338 ± 6[ab]	331 ± 3[ab]	291 ± 3[bd]
Sodium *mM*	142 ± 1	137 ± 1[a]	137 ± 2[a]	140 ± 1	143 ± 1	139 ± 1[a]	139 ± 1	138 ± 1[a]
Potassium *mM*	5.0 ± 0.2	4.8 ± 0.2	4.8 ± 0.3	4.0 ± 0.3[a]	4.4 ± 0.2	3.6 ± 0.2[ab]	3.1 ± 0.1[ab]	2.8 ± 0.1[abd]
Total CO_2 *mM*	24 ± 1	22 ± 1	22 ± 1	23 ± 2	26 ± 1[b]	11 ± 1[ab]	10 ± 1[ab]	20 ± 1[ad]
Chloride *mM*	106 ± 1	97 ± 1[a]	97 ± 2[a]	104 ± 2[c]	106 ± 2	112 ± 2[b]	110 ± 2[b]	106 ± 1[d]
β-OH-Butyrate *mM*	ND	ND	ND	ND	ND	3.7 ± 8[ab]	3.6 ± 6[a]	0.6 ± 0.2[d]
pH	7.44 ± 0.01	7.40 ± 0.02	7.37 ± 0.02	7.49 ± .03[c]	7.40 ± 0.01	7.08 ± 0.05[ab]	7.07 ± 0.03[ab]	7.39 ± 0.02[bd]
PA CO_2 *mm Hg*	39 ± 1	41 ± 1	45 ± 2	33 ± 2	40 ± 2	35 ± 2[b]	36 ± 2[b]	34 ± 2
PO O_2 *mm Hg*	89 ± 2	85 ± 3	81 ± 8	87 ± 4	98 ± 3[b]	117 ± 7[ab]	113 ± 4[b]	109 ± 5[b]
Body weight *g*	315 ± 9	275 ± 9[a]	270 ± 4[a]	294 ± 5	327 ± 5	259 ± 6[ab]	247 ± 6[ab]	270 ± 6[ab]

[a] $P < 0.05$ versus control group
[b] $P < 0.05$ versus respective NKD group
[c] $P < 0.05$ versus NKD pretreatment group
[d] $P < 0.05$ versus DKA pretreatment group

an initial wet wt, then oven drying the brain tissue at 100°C for 48 hours, after which time it was re-weighed. Dried brain was then extracted for 48 hours in 0.75 N HNO_3 and sodium and potassium content determined by flame photometry (Model No. 943; Instrumentation Laboratories, Lexington, MA, USA). Chloride content was measured with a chloride ion-selective electrode (Corning Instruments, Corning, NY, USA).

The method for measurement of organic osmolyte content in brain has been described previously [26]. Frozen brain tissue was crushed under liquid nitrogen and lyophilized. Amino acid content of lyophilized brain tissue was then determined by high performance liquid chromatography after derivitization with phenylisothiocyanate (Pico Tag; Waters Corporation, Milford, MA, USA). Methionine sulfone served as the internal standard. The content of myoinositol, sorbitol, creatine and betaine was determined by HPLC utilizing a Sugar Pak II Column (Waters). Maltose was utilized as the internal standard for each sample.

Statistical analysis

Data are presented as mean values ± SE. Studies comparing groups within an experimental model (DKA or NKD) were assessed by one-factor analysis of variance with significance determined by the Scheffe F-test (Statview 512+ Brain Power, Calabasas, CA, USA). Comparison of two groups in different experimental models (DKA vs. NKD, or hypotonic vs. isotonic fluid) was performed by use of an unpaired Student's *t*-test. Significance was accepted at the $P < 0.05$ level.

Results

Experiment I: Treatment of non-ketotic versus ketotic diabetes

In Table 1, body wt and plasma chemistry data are given for nondiabetic control, untreated diabetic and treated diabetic groups for both non-ketotic and ketotic diabetic models. In the NKD model, significant hyperglycemia and volume depletion occurred in the absence of ketoacidosis. In untreated and pre-treatment NKD rats plasma glucose was 43.7 ± 2.2 and 41.6 ± 2.9 mM respectively. In DKA rats severe hyperglycemia and ketoacidosis developed, though plasma glucose levels in untreated and pre-treatment animals (31.1 ± 0.8 and 32.1 ± 1.5 mM) were

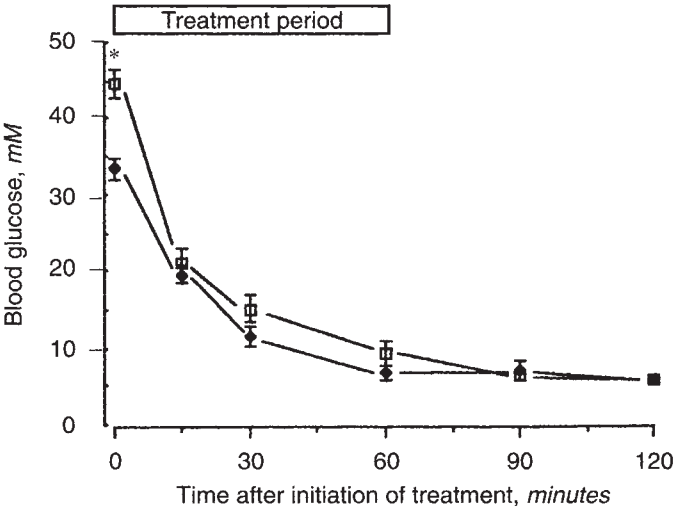

Fig. 1. *Course of plasma glucose with treatment of NKD (□) and DKA (♦) rats.* *$P < 0.05$ versus DKA.

significantly less than in NKD. This was associated with a significantly lower measured plasma osmolality prior to treatment in DKA than NKD.

In response to treatment with intravenous fluids and insulin, plasma glucose concentration decreased rapidly over the 60-minute treatment period and remained stable for 60 minutes following treatment (Fig. 1). Although plasma glucose values were higher in NKD than DKA rats prior to treatment, they fell to equivalent levels with treatment. There was no difference in the fall in plasma osmolality between models with a reduction of 42 ± 2 mOsm/kg in NKD rats versus 40 ± 3 mOsm/kg in DKA rats. Calculating the decrease in plasma tonicity using only sodium and glucose values also did not reveal a significant difference between groups. There was no significant change in plasma sodium following treatment in either NKD or DKA models and pre- and post-treatment plasma sodium levels were equivalent. In the DKA

Table 2. Brain content of water and inorganic solutes in NKD and DKA rats

	NKD			DKA		
	Control $N = 9$	Untreated $N = 10$	Treated $N = 9$	Control $N = 9$	Untreated $N = 11$	Treated $N = 11$
Brain water *liter/kg dry wt*	3.73 ± 0.03	3.65 ± 0.02	3.90 ± 0.02[ab]	3.75 ± 0.01	3.64 ± 0.02[a]	3.93 ± 0.02[ab]
Brain sodium *mmol/kD" dry wt*	192 ± 1	192 ± 2	182 ± 2[ab]	195 ± 1	201 ± 2	187 ± 2[b]
Brain potassium *mmol/kg dry wt*	446 ± 2	446 ± 2	443 ± 4	464 ± 2	468 ± 3	472 ± 5
Brain chloride *mmol/kg dry wt*	187 ± 7	175 ± 4	160 ± 4[a]	188 ± 6	200 ± 10	184 ± 8

[a] $P < 0.05$ vs. control
[b] $P < 0.05$ vs. untreated group

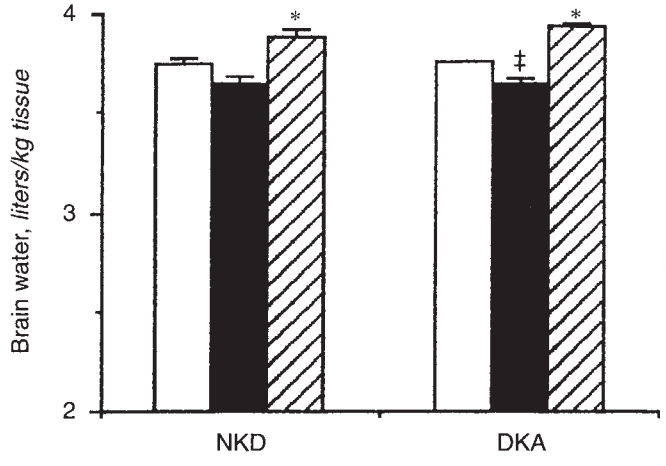

Fig. 2. *Comparison of brain water contents in NKD and DKA groups (*P < 0.05 vs. other groups, ‡P < 0.05 vs. non-diabetic control).* There was no significant difference between respective control (□), untreated (■), and treated (▨) NKD and DKA groups.

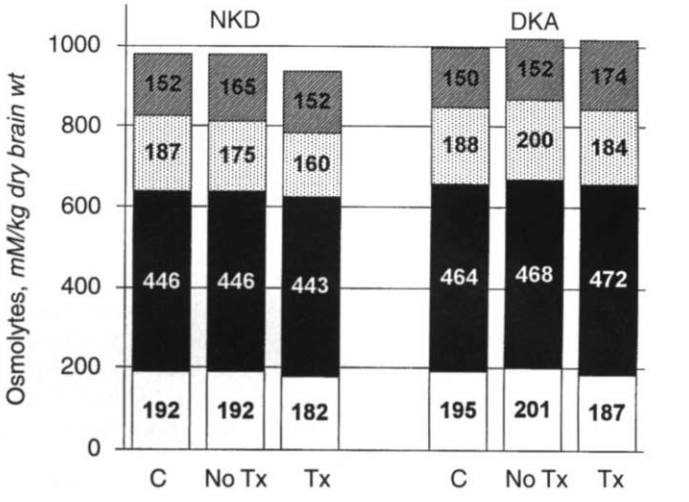

Fig. 3. *Contribution of organic and inorganic osmolytes to total brain osmolytes control (C), untreated (No Tx) and treated (Tx) groups in both NKD and DKA.* Symbols are: (▩) organic osmolytes; (▨) chloride; (■) potassium; (□) sodium.

rats, treatment reduced β-hydroxybutyrate concentration significantly. Metabolic acidosis also resolved with treatment in DKA rats and significant hypoxemia was not present in any group.

Brain water contents in nondiabetic control, untreated and treated diabetic groups for both NKD and DKA models are given in Figure 2. The decrease in brain water from 3.73 ± 0.03 liter/kg dry weight in controls to 3.65 ± 0.02 liter/kg dry weight in untreated NKD did not reach statistical significance. However, with treatment, brain water increased significantly to 3.90 ± 0.02 liter/kg dry weight. A similar pattern was found in DKA. Brain water content in untreated DKA was significantly less than that in nondiabetic control animals (3.64 ± 0.02 vs. 3.75 ± 0.01 liter/kg dry weight, $P < 0.05$). As in NKD, brain water content in DKA rats increased significantly with treatment to 3.93 ± 0.02 liter/kg dry weight. When comparing brain water content between NKD and DKA rats, there were no differences between nondiabetic control, untreated or treated groups (Fig. 2).

In both NKD and DKA models, brain sodium content fell following treatment, and brain chloride fell in NKD (Table 2 and Fig. 3). There were no significant changes in brain potassium content. Increases in brain organic osmolyte content were also not identified in uncontrolled NKD or DKA (Tables 3 and 4). After treatment, brain glutamine and taurine content increased significantly in DKA, but brain content of the summed total of major organic osmolytes was not significantly increased (Table 3 and Fig. 3).

Experiment II. Treatment of DKA with hypotonic versus isotonic fluid

To further explore the contribution of osmotic shifts to the development of cerebral edema in diabetic ketoacidosis, we compared brain water and electrolyte content in DKA rats treated with hypotonic versus isotonic replacement fluid. In Table 5, plasma values and body wt for these two groups are given. As in experiment I, severe hyperglycemia and metabolic acidosis was produced in both groups. The plasma glucose of 38.1 ± 2.1 mM and pH of 7.02 ± 0.04 in the isotonically-treated group did not differ from the plasma glucose of 37.8 ± 4.1 mM and pH of 7.07 ± 0.05 in the hypotonically-treated group. Normal arterial oxygenation was present in both groups throughout the study. In response to treatment, blood glucose values decreased at similar rates, reaching nearly identical values at 60 and 120 minutes (Fig. 4). Metabolic acidosis was also equivalently reversed in both groups. Plasma sodium concentration increased in the isotonic-treated group, but remained at pretreatment level in the hypotonic fluid-treated group. Correspondingly, plasma osmolality decreased less during treatment in the isotonic group than in the hypotonic group (55 ± 5 mOsm/kg vs. 71 ± 6 mOsm/kg, $P < 0.05$). Post-treatment brain water and electrolyte contents of

Table 3. Brain content of major organic osmolytes measured in NKD and DKA rats (expressed in mM/kg dry brain tissue wt)

	NKD			DKA		
	Control $N = 9$	Untreated $N = 10$	Treated $N = 9$	Control $N = 9$	Untreated $N = 11$	Treated $N = 11$
Myoinositol	18.0 ± 1.6	18.9 ± 1.3	17.2 ± 2.0	23.9 ± 0.8	24.1 ± 0.7	23.6 ± 0.5
Taurine	21.8 ± 1.4	26.0 ± 1.5	23.1 ± 2.0	21.3 ± 1.5	24.6 ± 2.0	28.9 ± 2.2[a]
Glutamate	51.6 ± 3.1	54.7 ± 3.2	48.7 ± 3.4	46.4 ± 3.7	46.9 ± 3.3	53.3 ± 3.1
Glutamine	23.3 ± 1.3	26.4 ± 1.8	26.8 ± 2.5	23.2 ± 1.7	23.0 ± 1.8	32.1 ± 2.2[ab]
Creatine	37.3 ± 3.7	39.6 ± 1.6	37.0 ± 4.1	34.9 ± 1.8	33.7 ± 0.8	34.9 ± 1.0
Total major organic osmolytes	152 ± 11.0	165 ± 4.5	152 ± 8.9	150 ± 6.6	152 ± 7.6	174 ± 8.4

[a] $P < 0.05$ vs. control group
[b] $P < 0.05$ vs. untreated group

Table 4. Brain content of urea, glucose, and minor organic osmolytes measured in NKD and DKA rats (expressed in mM/kg dry brain tissue wt)

	NKD			DKA		
	Control $N = 9$	Untreated $N = 10$	Treated $N = 9$	Control $N = 9$	Untreated $N = 11$	Treated $N = 11$
Urea	12.4 ± 0.9	18.9 ± 1.4[a]	17.7 ± 2.8	13.3 ± 0.9	22.9 ± 2.0[a]	16.9 ± 2.1[b]
Sorbitol	5.10 ± 0.6	6.29 ± 0.6	4.81 ± 0.8	5.09 ± 0.5	5.21 ± 0.3	3.58 ± 0.3[ab]
Glycine	3.52 ± 0.2	3.60 ± 0.2	3.50 ± 0.2	3.30 ± 0.3	3.73 ± 0.2	3.73 ± 0.3
Betaine	2.07 ± 0.2	1.95 ± 0.1	1.96 ± 0.2	1.05 ± 0.1[c]	1.17 ± 0.1	0.99 ± 0.1[c]
γ-Aminobutyric acid	9.25 ± 0.65	9.38 ± 0.55	8.41 ± 0.62	6.67 ± 0.50[c]	6.62 ± 0.46[c]	7.92 ± 0.48
Phosphoserine	8.45 ± 0.42	9.25 ± 0.78	6.69 ± 0.51[b]	8.68 ± 1.2	9.09 ± 0.80	7.07 ± 0.76
Phosphoethanolamine	6.27 ± 0.35	6.37 ± 0.30	4.91 ± 0.52[b]	5.33 ± 0.38	5.36 ± 0.30	5.79 ± 0.56
Serine	5.41 ± 0.37	4.88 ± 0.28	4.75 ± 0.37	4.78 ± 0.45	4.23 ± 0.28	4.91 ± 0.39
Alanine	4.42 ± 0.53	6.30 ± 0.81	5.41 ± 0.98	2.16 ± 0.21[c]	2.40 ± 0.21[c]	2.63 ± 0.21[c]
α-Aminobutyric acid	3.36 ± 0.33	3.51 ± 0.19	2.65 ± 0.33	2.93 ± 0.30	2.57 ± 0.24[c]	3.34 ± 0.43
Aspartic acid	2.44 ± 0.37	2.12 ± 0.29	1.97 ± 0.23	6.60 ± 0.81[c]	6.81 ± 0.45[c]	5.55 ± 0.76[c]
Threonine	2.26 ± 0.15	1.67 ± 0.10[a]	1.63 ± 0.19[a]	2.16 ± 0.21[c]	2.40 ± 0.21[c]	2.63 ± 0.21[c]
Other osmolytes[d]	3.45 ± 0.33	3.67 ± 0.33	3.63 ± 0.36	3.03 ± 0.24	3.05 ± 0.20	3.48 ± 0.34

[a] $P < 0.05$ vs. control group
[b] $P < 0.05$ vs. untreated group
[c] $P < 0.05$ vs. respective NKD group
[d] Sum of ethanolamine, valine, methionine, cystathione, isoleucine, leucine, arginine, tyrosine, β-alanine, and asparagine

Table 5. Plasma values and body weight in DKA rats treated with isotonic or hypotonic fluid repletion

	Hypotonic		Isotonic	
	pre-treatment $N = 9$	post-treatment $N = 9$	pre-treatment $N = 9$	post-treatment $N = 9$
Urea *mM*	19.2 ± 2.9	15.7 ± 1.7	23.1 ± 2.4	16.1 ± 1.7
Glucose *mM*	39.5 ± 5.1	6.7 ± 0.61[a]	38.1 ± 2.0	6.0 ± 0.6[a]
Osmolality mOsm/kg	372 ± 10	301 ± 3[a]	368 ± 8	312 ± 3[ab]
Sodium *mM*	142 ± 1.5	141 ± 1.3	141 ± 1.1	148 ± 0.8[b]
Potassium *mM*	3.4 ± 0.2	2.8 ± 0.1	3.5 ± 0.1	3.5 ± 0.2[b]
Total CO_2 *mM*	9 ± 1	20 ± 0.1[a]	11 ± 2	21 ± 2[a]
Chloride *mM*	110 ± 1.0	105 ± 2	114 ± 1.2	119 ± 1[b]
pH	7.07 ± 0.04	7.42 ± 0.02[a]	7.02 ± 0.04	7.36 ± 0.01[a]
PA CO_2 *mm Hg*	38.4 ± 3.3	30.5 ± 2.4	36.5 ± 2.4	34.3 ± 1.7
PA O_2 *mm Hg*	115 ± 8	107 ± 3	121 ± 7	115 ± 7
Weight *g*	237 ± 5	264 ± 5[a]	226 ± 6	252 ± 6[a]

[a] $P < 0.05$ versus pretreatment
[b] $P < 0.05$ versus hypotonic post-treatment

isotonic and hypotonic groups are shown in Table 6. Significantly less brain swelling occurred in animals treated with isotonic solution. Despite increased brain water, brain sodium content in rats treated with hypotonic fluid was significantly lower than those treated with isotonic fluid. There was no difference in brain potassium content between the two treatment groups.

Discussion

In the current study, we established an animal model of severe non-ketotic and ketotic diabetes mellitus in order to more clearly define the pathogenesis of cerebral edema that occurs after treatment of severe diabetes. The model controlled for the

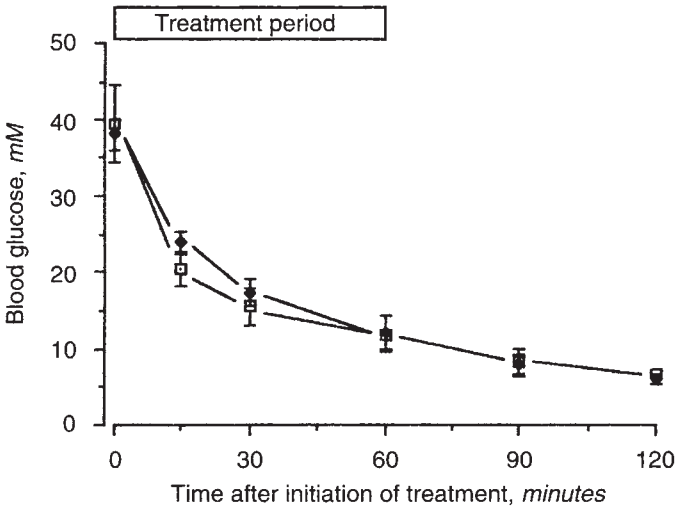

Fig. 4. *Course of plasma glucose in DKA rats treated with hypotonic (□) or isotonic (♦) fluid.*

presence or absence of acidosis so that its contribution to brain swelling could be assessed. Plasma sodium was maintained within normal limits after treatment of diabetes so that changes in brain water could be assessed independent of changes in plasma sodium. In our model, severe diabetes was present for five days prior to treatment, allowing adequate time for brain adaptation to the osmotic milieu. In contrast to previous studies, acute infusion of hypertonic glucose was not superimposed [18, 19]. Plasma values prior to treatment approximated human non-ketotic and ketotic diabetes. However, in animals with DKA, the increased anion gap and beta hydroxybutyrate concentration did not fully account for the decrease in plasma bicarbonate. Increased urinary losses of ketone bodies and a fall in plasma albumin, neither of which were measured in the study, may have contributed to this finding. Plasma osmolality and acidosis were corrected more rapidly (over two hours) than under normal clinical conditions in order to accentuate any differences between experimental groups. The study included measurement of brain organic osmolytes known to be involved in the adaptive response to osmotic perturbations [20, 21].

Rapid correction of hyperglycemia (Fig. 1) and hyperosmolality resulted in a significant increase in brain water content (Fig. 2). In both NKD and DKA, plasma osmolality decreased by about 12% with treatment. If the brain were a perfect osmometer and no solute were lost during treatment, an equivalent increase in brain water would be expected. However, the actual observed increase in brain water after treatment of NKD and DKA was only about 8%. Loss of brain electrolytes only partially accounts for the discrepancy between observed and predicted values. We assumed that during correction of hyperglycemia, glucose was also lost from brain; however, as discussed earlier, our experiments were not designed to measure these changes. Studies by other investigators, in which glucose was measured in tissue that had been frozen *in situ*, show that brain glucose concentration is about 30% of plasma values both before and after treatment of hyperglycemia [27]. If we assume that brain glucose content was similar in our studies, we would expect that brain glucose content would have decreased by approximately 25 to 35 mM/kg dry tissue weight, an amount that exceeds the measured loss of electrolytes. If brain glucose and electrolyte solute losses are taken into consideration, the calculated and actual increase in brain water after treatment of NKD and DKA are similar (**Appendix**). Thus, brain swelling after treatment of diabetes could be explained without invoking new solute formation ("idiogenic osmoles"). Consistent with these observations, we found no significant increase in total brain organic osmolytes after treatment of non-ketotic or ketotic diabetes. However, because of the limited sensitivity of our methodology, we cannot exclude small increases in total organic osmolytes that would be difficult to detect. Indeed, after treatment of DKA, total brain organic osmolytes did trend upward and the individual osmolytes taurine and glutamine increased. However, the total osmotic contribution of these changes has little impact on our conclusions.

Table 6. Brain content of water and inorganic solutes in ketotic diabetic animals treated with hypotonic or isotonic fluid repletion

	Hypotonic	Isotonic
Brain water (L/kg dry wt)	3.90 ± 0.02	3.78 ± 0.02[a]
Brain sodium (mmol/kg dry wt)	206 ± 3.3	215 ± 1.3[a]
Brain potassium (mmol/kg dry wt)	484 ± 2.7	486 ± 2.2

[a] $P < 0.05$ versus hypotonic group

Brain sodium decreased equivalently in both non-ketotic and ketotic groups after treatment. Rather than causing brain edema, changes in brain sodium served to militate against it. A similar decrease in brain sodium occurs when brain swelling is induced with hyponatremia. This adaptation is thought to be due to loss of sodium chloride from the brain via bulk flow of interstitial fluid into the cerebrospinal fluid [28]. Our findings differ from those of Arieff, who found that brain sodium increases in animals that develop cerebral edema after treatment of severe hyperglycemia [18, 19]. It has been hypothesized that stimulation of Na^+/H^+ exchange (via insulin or correction of acidosis) or increased blood-brain barrier permeability to sodium induced by hyperglycemia may predispose to cerebral edema [13]. If this were the primary event, brain swelling would be expected to be associated with an increase in brain sodium. In contrast, our findings imply that the primary pathogenic mechanism for brain swelling was not driven by sodium influx into brain. Presumably, it was driven by influx of water to brain cells that had retained additional solute while adapting to hyperglycemia.

The present studies compared animals with diabetic ketoacidosis treated with hypotonic and isotonic fluid in an attempt to more directly assess the contribution of osmotic change to the pathogenesis of the cerebral edema observed. Brain water in animals treated with isotonic fluid (Table 6) was similar to non-diabetic controls and higher than in untreated DKA (Table 2), suggesting that a limited increase in brain water did occur with its use. However, treatment with hypotonic fluid decreased plasma sodium and osmolality to a greater degree and resulted in significantly more brain swelling than in animals treated with isotonic fluid, despite lower brain sodium content. The differences in plasma osmolalities in the two groups can account for the differences in brain water. Assuming equality of brain and plasma osmolality, total brain solute after treatment in the hypotonic group would equal plasma osmolality × brain water content (301 mOsm/kg × 3.90 liter/kg brain tissue = 1174 milliosmoles); in the

isotonic group, total brain solute is similar to the hypotonic group (312 mOsm/kg × 3.78 liter/kg brain tissue = 1179 milliosmoles). The result confirms the predominant effect of a rapid decrease in plasma osmolality in the pathogenesis of the cerebral edema observed in our experiments: since isotonic fluid minimized brain swelling despite use of insulin, correction of acidosis, and presumably similar lowering of oncotic pressure in both groups, these factors do not appear to have played a major role in the brain swelling observed. Moreover, brain sodium content in the hypotonic group was significantly lower than in the isotonic group, indicating that the cerebral edema was not due to sodium influx into brain. Rather, sodium efflux from brain occurred, presumably as an adaptive response to cerebral edema.

It may seem surprising that brain water did not rise above normal levels when ketoacidosis was treated with isotonic fluid even though the increase in plasma osmolality due to an increase in plasma sodium (approximately 14 mM compared to hypotonic fluid therapy) failed to match the decrease in plasma osmolality due to a falling blood sugar (approximately 30 mM). However, it should be noted that after treatment with both hypotonic and isotonic fluid, brain water increased in comparison to untreated diabetes.

Our findings do imply that the routine use of hypotonic fluid replacement in DKA after obtaining hemodynamic stability with isotonic fluid be reconsidered. Currently, the general goal of such treatment is to maintain serum sodium at pretreatment levels while decreasing plasma osmolality by correction of hyperglycemia. Clearly, the results of the present study cannot be extrapolated to the clinical arena. However, treatment regimens that allow the serum sodium level to increase initially, to offset the osmotic effect of the decrease in blood glucose, may be desirable.

Cerebral edema was equivalent after treatment of both non-ketotic and ketotic diabetic animals, indicating that the presence and correction of severe diabetic ketoacidosis in the present experiments were not major factors predisposing to brain swelling (Fig. 2). However, the experimental findings do not completely exclude a contribution of acidosis or its correction to the increase in brain water. Pretreatment plasma glucose was about 10 mM less in acidotic versus non-acidotic animals, and thus more brain swelling in acidotic animals might have been observed if pretreatment plasma glucose had been equivalent between groups. However, both the plasma osmolality and plasma tonicity (calculated contribution of sodium and glucose to osmolality) decreased to the same degree after treatment in non-ketotic and ketotic groups. The brain content of taurine and glutamine increased after treatment of ketotic diabetes, though the increase in total major brain organic osmolytes was not significant. Nonetheless, the differences in brain organic osmolyte content after treatment of non-ketotic and ketotic diabetes deserve further investigation. Our studies do not explain the apparent predisposition to cerebral edema in DKA in the clinical setting. Possibly, younger patients, who have a higher incidence of DKA than adults, are more susceptible to brain swelling.

Although our studies could account for the increase in brain water that occurs after treatment of diabetes, we could not account for the brain's resistance to dehydration in untreated diabetes. In the present study, plasma osmolality of untreated diabetic animals increased by approximately 12%, due to hyperglycemia. If we assume, as discussed previously, that brain glucose concentration is 30% that of plasma, a 12% increase in plasma osmolality due to hyperglycemia would be associated with a 4% increase in brain glucose concentration. Thus, if the brain were a passive osmometer, an 8% decrease in brain water would be expected. In fact, however, brain water in non-ketotic and ketotic diabetes was only 2% lower than controls, and this difference reached statistical significance only in the ketotic group. The present study cannot explain this observed adaptation to the hyperosmolality on the basis of an increase in brain solute. In our study, in contrast with other investigators, no significant change in brain electrolyte or organic osmolyte content was found in either non-ketotic or ketotic diabetes [22, 24]. Thus, our results indicate that the organic osmolytes which function to preserve brain water in hypernatremia do not serve this role in hyperglycemia. We cannot exclude an increase in other unidentified osmolytes, or an increase in the osmotic activity of known osmolytes.

In summary, treatment of both non-ketotic and ketotic animals resulted in a nearly equivalent degree of cerebral edema with no change in total measured osmolytes (although brain glutamine and taurine increased after treatment of ketotic animals). Cerebral edema could be attributed to the decrease in plasma osmolality during correction of hyperglycemia, and the presence of acidosis appeared to play a minor role. Despite the cerebral edema induced in our studies, brain sodium decreased, demonstrating that the brain swelling was not due to a process resulting in sodium influx into the brain. Treatment of ketotic hyperglycemic animals with isotonic saline conferred significant protection against cerebral edema, confirming the dominant influence of changes in plasma osmolality.

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Appendix: Predicted brain water in treated NKD and DKA

Assuming that the brain and plasma are in osmotic equilibrium, the change in brain water (BW) can be predicted by the measured change in plasma osmolality (P_{Osm}) and brain solute (B_{Sol}) before and after (pre and post) treatment of diabetes using the following relationship:

$$\frac{P_{Osm}\,(pre)}{P_{Osm}\,(post)} = \frac{B_{Sol}\,(pre)/BW\,(pre)}{B_{Sol}\,(post)/BW\,(post)}$$

and thus,

$$\frac{BW\,(post)}{BW\,(pre)} = \frac{P_{Osm}\,(pre)\,B_{Sol}\,(post)}{P_{Osm}\,(post)\,B_{Sol}\,(pre)}$$

At osmotic equilibrium, brain solute content equals the product of plasma osmolality and brain water content. If brain electrolytes were the only solutes to change during treatment of diabetes, then

$$B_{Sol}(post) = [P_{Osm}(pre) \times BW\,(pre)] + (\Delta \text{ brain electrolyte content})$$

Thus,

$$\frac{BW\,(post)}{BW\,(pre)} = \frac{[P_{Osm}\,(pre) \times BW\,(pre)] + (\Delta \text{ brain electrolyte content})}{P_{Osm}\,(post) \times BW\,(pre)}$$

A predicted value for post-treatment brain water can be calculated from the pretreatment and post-treatment plasma osmolality values and the measured pretreatment and posttreatment brain electrolyte content. This calculation provides the change in brain water that would be predicted if only brain electrolytes were to change during treatment. Based on the above assumptions, the actual and predicted increase in brain water for NKD (7.2 ± 1.2% vs. 9.7 ± 1.3%, P = NS) and DKA (8.0 ± 0.5% vs. 11.4 ± 1.0%, $P < 0.05$) indicate that the changes in plasma osmolality and brain electrolytes alone are more than enough to account for the measured brain water changes.

The above estimation, however, assumes no change in brain glucose with treatment. Because an accurate determination of brain glucose requires *in situ* freezing of brain tissue, (a method that would have sacrificed precision in our measurement of brain water) this analysis was not made in the present studies. Based on studies of other investigators in which brain glucose was determined in diabetic rats after *in situ* freezing of brain tissue, brain glucose concentration can be assumed to equal 30% of the plasma value [27]. Using this assumption, a prediction of brain water based on changes in brain electrolyte and glucose content can be calculated from the following equation:

$$\frac{\text{BW (post)}}{\text{BW (pre)}} =$$

$$\frac{[\text{POsm (pre)} \times \text{BW (pre)}] + (\Delta \text{ brain electrolyte content} + \Delta \text{ brain glucose content})}{\text{POsm (post)} \times \text{BW (pre)}}$$

Based on changes in both brain glucose and electrolytes, there is less difference between the actual and predicted increase in brain water for NKD (7.2 ± 1.2% vs. 7.5 ± 1.3%, NS) and DKA (8.0 ± 0.5% vs. 8.5 ± 2.6%, NS) than if only brain electrolytes were assumed to have changed.

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